CLEAN COPY OF SPECIFICATION

Figure 8 illustrates another tube design having an overall length of 0.197 inch, a wall thickness of 0.0035 inch, a diameter of 0.041 inch, and a rectangular opening 0.118 inches in length.

Figure 9 depicts yet another tube design having a length of 0.197 inch, wall thickness of 0.035 inch, diameter of 0.041 inch, and a rectangular opening 0.197 inches in length.

Figure 10 depicts another tubular design having a length of 0.197 inch, a diameter of 0.41 inch (overall), wall thickness of 0.035 inch, and two rectangular holes 0.039 inch in length.

Figure 11 depicts another bottle-like tubular design having an overall length of 0.197 inch, diameter of 0.041 inch (large portion), wall thickness of 0.035 inch, rectangular opening that is 0.039 inch long and a circular opening 0.020 inch in diameter.

Figure 12 depicts another bottle-like tubular design having an overall length of 0.197 inch, a diameter of 0.041 inch, wall thickness of 0.03 S inch, and two round holes that are 0.020 inch in diameter.

Seeds with different types of perforations allow drugs to be released at different rates, e.g., rectangular holes can be used to release chemotherapeutic drugs to be released intratumorally at a fast rate. Spherical/circular holes can be used to deliver biologics at a relatively slow rate at the tumor site. The subject seeds, which are alternatively referred to as "GENESEEDS®", can also be filled with a cocktail of drugs containing genetic drugs (viruses, plasmids, etc.), chemotherapeutic drugs, radionuclides, toxins, cytokines,

The efficacy of the subject hollow seed delivery system for delivering a therapeutic moiety, e.g., nucleic acid sequence, will be confirmed in xenograft animal models. For example, mice will be implanted with human tumors, such as breast cancer, and squamous carcinoma, and then treated with seeds according to the invention that comprise a nucleic acid sequence or a cytokine and a source of ionizing radiation.

The above-described novel therapeutic device will now be described in the following example. It should be understood that the invention is not limited to the specific embodiments described above or to the example as set forth below, but is defined by the following claims in light of the description herein.

EXAMPLE

A. Seed Design

The first step in this process is to optimize seed design to satisfy identified clinical needs. Although we have made some prototype seeds, variables include seed size, shape, and number of holes to provide portals for diffusion. Batches of 200 seeds will be manufactured for described experiments in an animal tumor model.

The prototype GENESEED® consists of a metallic tube made of high purity titanium metal suitable for medical applications with a thickness of 0.005 inch. Low weight, high strength titanium is the metal of choice for the majority of implantable devices. Titanium grade metal specified in the American Society for Testing of Materials F67-69 "Standard Specifications for Unalloyed Ti for Surgical Implant Applications" will be used. Titanium of the same grade has been in use in surgical implants for interstitial treatment

special transfer devices. These transfer devices are aluminum blocks of rectangular shape suitable for freezing at -70°C. These blocks contain small holes for the storage of GENESEEDS® (Figure 2).

GENESEEDS® will function as delivery devices to freeze the biological material and transfer it to the hospital in the frozen state until ready for use in patients. If needed, the GENESEEDS® can be placed in specially fabricated metallic cartridges and kept at very low temperatures. Seed cartridges for storage of radioactive seeds are already available in the brachytherapy industry and these cartridges can be modified for low temperature applications.

B. Seed Manufacture

High purity titanium tubes (medical grade metal) are cut to required size (± 3%). The seeds will then be washed with an aqueous solution containing a mild detergent followed by acetone and sterile water for injection. The washed seeds will be dried in an oven at 110 ° C for about two hours. Autoclaving will be performed to assure sterility. The seeds will be allowed to cool to room temperature. The viral solution will be added to the seed, using specially designed transfer devices which are adaptable to robotic control. The transfer device containing GENESEEDS® will be kept frozen at –70°C until ready for use in animals. Small numbers of seeds can be prepared manually for initial preclinical studies. Once a suitable configuration is identified, large scale manufacturing of GENESEEDS® can be performed employing the proprietary technology developed by Best Industries Inc. and is currently in use for the production of iodine and palladium

mice(Balb/c) and non-human primates (aotus). Conditionally-replicating herpes viruses are novel vectors ideally suited for this innovative form of prostate cancer therapy. A Phase I study of 6207 is now being completed which demonstrates that this conditionally-replicating herpes vector can be inoculated directly into the human brain at titers as high as 3x109 pfu without neural or systemic toxicity. A phase II trial of 6207 for malignant gliomas is now being planned. We anticipate that within this next year an IND for human trials of intraprostatic inoculation of 6207 to treat post-radiation local recurrences will be filed. The studies designed herein may extend this concept to allow more accurate delivery of the vector within prostatic, brain, or other tumors and tissues.

D. Experiments

1. Optimization of the design of GENESEEDS® for interstitial delivery of viral vectors and cytokines.

The four different types of GENESEEDS® described in Figure 1 will be filled with viral solutions and frozen at -70°C. The seeds will be implanted interstitially in mice bearing tumor xenografts (prostate tumor models). Melting and release of viral solution occurs rapidly. At selected time points post implantation, the animals will be sacrificed and the tumor will be excised. The extent of diffusion and virus entry into tumor cells will be evaluated using histochemisfiry. The optimum design will slowly diff-use the viral material, allowing maximal intracellular viral uptake in tumor cells.

interstitial injection, however, animal organs including lungs, liver, and brain will also be sectioned and scored.

EXPERIMENT 1

Specific Aim I will be addressed with the following experiment.

Evaluation of viral distribution within tumors as a function of time after GENESEEDS® implant

Time								
		0	4h	12h	24h	2 days	3 days	7 days
Controls (buffer only, all designs		X			X			X
Controls, intratumoral injection		X	X	X	X	X	X	X
GeneSeed, Design	A	X	X	X	X	X	X	X
	B	X	X	X	X	X	X	X
	C	X	X	X	X	X	X	X
	D	X	X	X	X	X	X	X

Three mice will be used per time point. Controls and design A seed experiments will be performed for all time points in the initial experiment. Based on resultant data, designs B, C, and D will be studied at the most relevant time points after implantation. This strategy should reduce the necessary total number of mice. Similarly, controls will also be performed with designs B, C, and D seeds at selected time points.

Anticipated Results

Non-replicating vectors would be expected to be maximally distributed at early time points. Since 6207 is a conditionally replicating vector, maximal distribution is anticipated at later time points. Our experimental plan will be modified accordingly once

design A test samples and controls are examined. These experiments will only use 1 seed per tumor, with the expectation that multiple seed use in a tumor will similarly depend on optimal single seed design for viral release. The seeds will be loaded with 10^6 pfus per seed. The controls include seeds with buffer only, as well as direct injection of Viral solution into the tumor. Comparisons of patterns of distribution will be made.

EXPERIMENT 2

Specific Aim II will be addressed with the following experiment.

Tumor growth delay

The optimal seed design based on data from experiment #1 will be used in tumor growth delay studies

1. Controls #1	Tumor bearing mice
2. Controls #2	PBS in seeds
3. Controls #3	Viral, direct intratumoral injection
4. GeneSeed	(optimal design) with virus

Injections will be performed into - 120-150 mm^3 tumors as described. Eight mice will be used for each experimental group. Animals will be monitored for 30 days and tumor volume will 3 be plotted as a function of time. Animals will be sacrificed, on day 30 or when the tumor volume exceeds 1 cm^3.

Anticipated results/interpretation of data

We anticipate tumor growth delay to occur in GENESEEDS® and direct intra-tumor injected animals. If needed, additional experiments will be performed using more than

one seed per tumor. The observation of tumor growth delay comparable to direct tumor injection will be the endpoint confirming the utility of GENESEEDS® for viral vector delivery. Improved distribution experiments to show GENESEEDS® superiority over direct injection may require larger tumors in a large tumor model system and may be considered in a Phase II proposal.

Methodology

Cell Lines: LNCaP cells are maintained in IMEM containing 5% calf serum at 37°C in 5% $C0_2$ with penicillin and streptomycin added to all media, and are tested to ensure freedom from mycoplasma contamination.

Subcutaneous Tumor Model: All animal procedures require approval by the Georgetown University Animal Care and Use Committee. The mice (6-to-7 week old male BALB/c nu/nu for human tumors) are anesthetized with an i.p. injection of a 0.25 - 0.30 ml solution consisting of 84% bacteriostatic saline, 10% sodium pentobarbital (1 mg/ml: Abbott Laboratories , Chicago, IL) and 6% ethyl alcohol or inhalation of 2-3 minimal alveolar concentration of methoxyflurane. LNCaP tumors are induced by s.c. flank injection of $5x10^6$ LNCaP cells in 0.1 ml with an equal volume of Matrigel and LNCaP cells in suspension. Tumors are measured by external caliper to the 0.1 mm, and volumes are calculated (V=H x L x W). Once a tumor volume of approximately 120-150 mm 3 is reached, tumors are either inoculated with 5-10 ,ul containing 10^7 plaque forming units (pfu) 6207 or virus buffer (150mM NaCl, 20mM Tris, pH 7.5). Experiments using seeds may require the placement of 1-2 GeneSeeds to deliver a comparable number of